

Intravenous Perfluorocarbon After Onset of Decompression Sickness Decreases Mortality in 20-kg Swine

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Introduction: Decompression sickness (DCS) occurs when bubbles form due to pressure decreases with severity ranging from trivial to fatal. Standard treatment requires a hyperbaric chamber, not likely to be available at remote sites or during a disabled submarine escape or rescue. Alternative (non-recompressive) treatments are needed. Intravenous administration of emulsified perfluorocarbons (PFCs) enhances oxygen delivery to, and inert gas removal from, tissues. Swine studies show PFCs administered with supplemental oxygen before symptom onset can decrease DCS incidence. We used a swine model to test whether PFC plus supplemental oxygen could improve outcome when infused after DCS symptom onset. **Methods:** After rapid decompression from 31 min at 200 fsw (7.06 ATA) animals were observed for signs of DCS. Upon DCS onset animals received 100% O₂ and were randomized to receive either saline or PFC. Oxygen administration was continued for 1 h and the primary outcomes of mortality and/or abnormal gait were noted 24 h after surfacing. **Results:** PFC significantly improved survival, with 18/25 (72%) PFC treated animals and 13/29 (45%) saline treated animals alive at 24 h post-exposure. Objective measures of stance/gait trended toward improvement; spinal cord lesions correlated with severity of stance/gait abnormalities. **Conclusion:** PFC administered after DCS onset improved survival in this 20-kg swine model. Further study into the mechanisms of benefit and delayed DCS therapy are warranted.

Keywords: perfluorocarbon emulsions, decompression illness, hyperbarics, oxygen.

WHEN AMBIENT PRESSURE is significantly decreased, inert gas in tissue and blood may form bubbles. Intravascular bubble formation leads to tissue ischemia, endothelial damage, inflammation, and complement and clotting activation (15). Though not completely understood, decompression sickness (DCS) is likely the result of bubble formation and these various cascading effects.

DCS manifestations range from simple joint pains to severe injury, including cardiopulmonary failure, neurologic deficits, or death. Cardiopulmonary DCS leads to right-sided heart failure and pulmonary edema (2,5). Neurologic injury from DCS is less clear, but may result from either local bubbles damaging tissues, arterIALIZED bubble(s) causing ischemia, or poor venous return leading to engorgement and infarction (16,18,19).

Standard DCS therapy is centered around decreasing bubble size with increased ambient pressure, enhancing inert gas elimination, and increasing oxygen delivery to compromised tissue beds. However, sophisticated hy-

perbaric chambers and oxygen delivery assets may not be readily accessible, leading to delayed therapy that may increase morbidity (33). Such delays could be considered inevitable in disabled submarine (DISSUB) rescue efforts or while diving in remote areas.

Alternative therapies are needed to treat DCS when a hyperbaric chamber is not readily available. So called “non-recompressive therapies” for DCS have generally been disappointing (24). However, one potential therapy appears promising: perfluorocarbons (PFCs). Developed as part of the Manhattan Project during World War II to serve as inert insulating material, PFCs are synthetic “oils” made up of polyfluorinated carbon chains. It was later discovered in the mid-1960s that PFCs could dissolve and transport large amounts of non-polar gases, including O₂ and N₂. While not hydrophilic, PFCs combined with emulsifying agents will dilute in aqueous solutions such as blood plasma. PFCs have a very high capacity for dissolving respiratory gases. The O₂ solubility of PFC is 20–25 times greater than that of blood plasma and may approach 60 volume % at 1 ATA (whole blood carries 20 volume %). The carrying capacity of PFC emulsions for N₂ may approach 50 volume % at 1 ATA (plasma N₂ solubility is 0.015 volume %) (9). Dissolved gases are not chemically bound by PFC compounds and both dissolve into and come out of solution in a linear fashion based on partial pressure. The increased and linear solubility of respiratory gases in PFC emulsions make them ideal candidate compounds for reducing DCS risk and severity through elimination of N₂ and improved O₂ delivery. Additionally, PFCs have been shown to decrease bubble adhesion to the endothelium via their surfactant properties.

All of these factors make intravenous PFCs a likely candidate for non-recompressive therapies for DCS. In

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fact, PFCs have shown survival benefits in animal models of DCS when used prior to the onset of symptoms (9,22) and gas embolism (28). For the study to be described here we used Oxycyte, a third-generation 60 volume % PFC emulsion procured from Oxygen Biotherapeutics International (Costa Mesa, CA) to examine whether PFC administered after DCS onset would be an effective treatment therapy.

METHODS

The methods reported were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1996. Before commencing, our Institutional Animal Care and Use Committee reviewed and approved all aspects of this protocol. The institutional animal care facility is fully AAALAC accredited and the veterinary staff members are familiar with our 20-kg swine model.

Our laboratory designed the swine model for DCS, demonstrating that DCS incidence increases from 25 to 75% over a range of 87–112 fsw (3.64–4.39 ATA) (10). We selected a rapid compression/decompression profile known to reliably produce a high incidence of severe DCS (9). Swine were randomized to receive either intravenous PFC or saline along with supplemental oxygen. Outcomes of death, gait abnormality, and spinal cord histology are reported.

Animals

Male Yorkshire swine ($N = 54$) from a single vendor (Thomas Morris Inc., Reisterstown, MD) were housed in free running cages at our animal care facility where they acclimated for 5 d prior to any procedures. They were fed standard pig chow twice daily (2–2.5% bodyweight; Quality Lab Prod, Elkridge, MA) with free access to water.

Treadmill Training and Use

Spinal cord DCS is manifested as paresis/paralysis and sensory deficits (1). A reliable indicator of hind limb function is the swine's ability to walk. We incorporated the Tarlov Scale, the recognized standard developed specifically for spinal cord pathology in swine (31). Animals were trained to walk on a treadmill (T-2000, GE Healthcare, Milwaukee WI) in three sessions starting at least 2 d before hyperbaric exposure. Each session was complete when the animal walked comfortably for 5 min at 1 mph, but never exceeded 15 min. Normal gait was defined as walking at 1 mph for 5 min.

Ear Vein Catheterization

On the day prior to hyperbaric exposure, animals were placed in a Panepinto sling and sedated with diazepam (intramuscular, 0.25 mg · kg⁻¹; Abbott, North Chicago, IL). An ear vein was catheterized with an 18-gauge 2-in angiocatheter and secured with tape. The

animal recovered comfortably in the sling until fully awake and able to ambulate.

Hyperbaric Exposure

On the day of the dive, awake, unanesthetized swine were lead into a transport kennel (22" x 32" x 22", Vari-Kennel, R.C. Steele, Brockport, NY) that was then positioned inside the hyperbaric chamber (45 cu ft). Viewports were fitted with cameras aligned to observe and record the animals throughout the hyperbaric exposure. The chamber was sealed and pressurized using ambient air.

Based on pre-study work-up and our familiarity with this model (9), the animals underwent a nonlinear compression profile to 200 fsw; 0–36 fsw at 10 fsw · min⁻¹, 37–69 fsw at 20 fsw · min⁻¹, 70–103 fsw at 30 fsw · min⁻¹, and 104–200 fsw at 60 fsw · min⁻¹. Bottom time was defined as the time from leaving surface pressure until time leaving bottom pressure. After 31 min of bottom time, decompression was initiated at 30 fsw · min⁻¹ until surface pressure was reached and the chamber door opened. It was expected that this profile would result in minimal barotrauma and 60% incidence of death or paralysis due to DCS.

Immediate Post-Dive Procedures

The animals were taken out of the chamber, removed from their kennels, and placed in a Panepinto sling. The ear vein catheter was used to administer 0.25 mg · kg⁻¹ diazepam and animals were observed for signs of cutis marmorata ("skin bends") as previously described (3). Skin bends have been shown to reliably precede the onset of severe DCS in 20-kg swine (1).

Treatment

At the onset of cutis marmorata, a photograph of the lesion was obtained and the principle investigator left the area. The animal was then given 100% O₂ by snout cone (Smith Medical North America, Wausesha, WI) and randomized to receive either 5 cc · kg⁻¹ intravenous PFC or an equivalent dose of normal saline. The animals were continuously observed for signs of distress, including thrashing or vocalization, which were treated with additional diazepam (0.125 mg · kg⁻¹ up to 2 mg · kg⁻¹). During the hour observation, oxygen saturation and pulse were measured by tail pulse oximetry (Oxisensor II, Nelcor Puritan Bennett, Pleasanton, CA). Any deaths were confirmed by the principle investigator. After 1 h the nose cone was removed and the animal returned to the holding pen. Animals were then intermittently observed for pain and comfort.

24-h Assessment

The animals were assessed for their ability to stand 24 h (± 2 h) post-dive. If able to stand the animal was assigned a Tarlov score of 3 and placed onto the treadmill. Treadmill speed was then gradually increased in 0.2-mph increments over approximately 30 s until the animal was able to walk at 1 mph. This earned a Tarlov score of 5. If the animal was able to walk but unable to

achieve 1 mph, the subject was scored a Tarlov 4 (weak walk). Other Tarlov scores were assigned as: complete paralysis of hind limbs - 0; minimal movement of hind limbs - 1; able to stand with assistance only - 2. Animals with a Tarlov ≥ 3 were returned to their holding pens and assessed for limb function again at 7 d post-dive, then underwent perfusion fixation. Animals with a Tarlov < 3 underwent perfusion fixation at 24 h. All animals that survived the initial 1-h observation period underwent perfusion fixation of the spinal cord at either 24 h or 7 d, based on their functional status as described.

Perfusion Fixation

Heparin ($100 \text{ IU} \cdot \text{kg}^{-1}$; APP Pharmaceuticals, Schaumburg, IL) was administered intravenously prior to euthanasia. Animals were then euthanized by intravenous administration of $0.1\text{--}1.5 \text{ mL} \cdot 10 \text{ kg}^{-1}$ Euthasol. After confirmation of death, the heart was exposed via thoracotomy and a large-bore cannula placed in the left ventricle. A second cannula was placed in the right ventricle to aid in draining fixative. One liter of heparinized 0.9% saline was infused, followed by 5–7 L of 10% buffered formalin. Following perfusion fixation, the spinal column was cut, the spinal cord removed, and then placed into 10% buffered formalin.

Histology

The spinal cords were processed by an independent facility (Charles River Pathology Associates, Frederick, MD). Trimmed at levels C5-6, T8-9, and L3-4, cord sections were processed through graded alcohols, embedded in paraffin, sectioned at $5 \mu\text{m}$, and stained with hematoxylin and eosin. Sections were scored by a pathologist blinded to the therapy received. Scoring of changes was based on a 1-4 scale: minimal change - 1; mild change - 2; moderate change - 3; marked change - 4. If no changes were evident the section was called normal. Axonal degeneration, hemorrhage, and mononuclear cell infiltration were scored in the white matter; gray matter and pia were scored only on hemorrhage and mononuclear cell infiltration.

Statistics

Survival and gait: Pre-dive weights were compared by Welch's two-sampled *t*-test. The primary end-point (proportion of animals that survived 24 h) was evaluated by a Fisher's exact test and a two-sample, one-sided test for equality of proportions. Animals with a Tarlov < 3 or death were compared against those with a Tarlov of ≥ 3 by Fisher's exact test.

Histology: Animals with Tarlov < 3 were euthanized at 24 h, so it was not possible to compare treatment groups. Pathology scores were summed within each region and displayed by Tarlov score and region at 24 h using a box plot.

RESULTS

Of the 54 animals studied, 25 received PFC and 29 received normal saline solution (NSS); two mis-

randomizations occurred. Mean weights between groups were not significantly different (Fig. 1). For the animals treated with PFC 18/25 (72%) survived with 13/29 (45%) surviving in the NSS group. Similarly, at 24 h, 13/25 (52%) PFC animals scored > 3 on Tarlov versus 8/29 (28%) given NSS. A one-sided Fisher's exact test for survival at 24 h between the PFC and NSS groups was significantly different, favoring PFC therapy ($P < 0.05$; confidence interval 1.056–11.71). A two-sample one-sided test for equality of proportions with continuity correction also showed significant differences between PFC and NSS ($\chi^2 = 3.019$, $\text{df} = 1$; $P < 0.05$).

A one-sided Fisher's exact for the combined endpoint of a Tarlov ≤ 3 at 24 h (including death) compared to survival yielded a difference approaching significance ($P = 0.059$), suggesting that some surviving animals had significant residual neurologic deficits (Fig. 1). Of the spinal cord lesions, white matter was the most widely affected area (Fig. 2). Abnormalities noted were hemorrhage, mononuclear cell infiltration, and axonal degeneration.

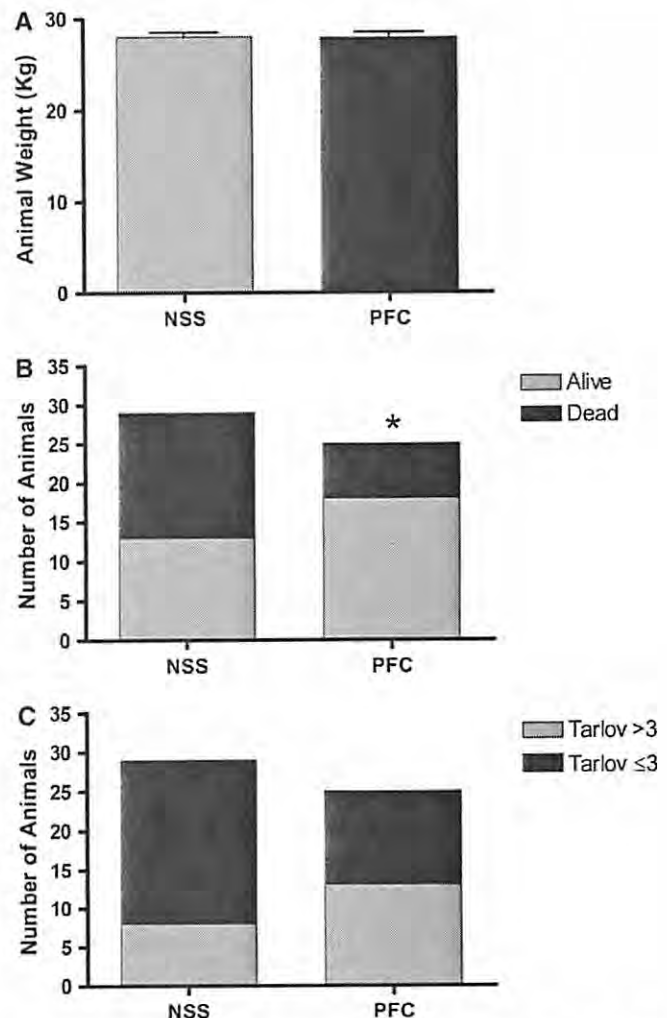


Fig. 1. A) Mean weights between treatment groups. B) Animal survival in the PFC group compared to controls. * A significant increase ($P < 0.05$) in survival in the PFC group vs. the controls. C) Tarlov scores in the PFC and control groups.

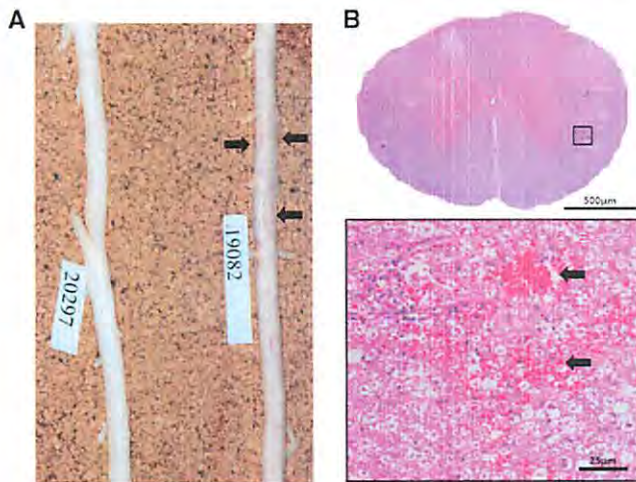


Fig. 2. Spinal cord pathology following DCS in swine. A) Gross observation of spinal cords extracted from animals with severe DCS (19082) compared to animals without severe DCS (20297) demonstrate diffuse hemorrhagic injury. B) Microscopic examination of the of hemoxilin/eosin stained coronal sections of DCS injured cords reveal hemorrhagic infiltration of the white matter (arrows in inset). There is also evidence of axonal degeneration, which presents as areas of diminished staining in the low power coronal section.

DISCUSSION

To our knowledge this is the first study demonstrating a survival benefit when intravenous PFC along with O_2 was used as treatment after the onset of DCS. Clark first demonstrated the enhanced oxygen carrying capacity of neat PFC in 1966 by submerging a mouse in oxygenated PFC for several hours (6). In the neat state PFCs are immiscible in water and need to be emulsified for intravenous use. In the emulsified state PFC can dissolve large amounts of oxygen. Their enhanced O_2 solubility also facilitates the transport of O_2 bound to Hgb to tissue (27).

The average particle diameter of PFC emulsions is about $0.2\ \mu\text{m}$, compared to $5\text{--}7\ \mu\text{m}$ for RBCs. Small particle size and enhanced O_2 transport are likely the mechanisms responsible for PFC-enhanced tissue oxygenation in constricted microcapillaries too small for RBCs to pass (12), improved oxygen delivery to microcirculation as demonstrated in hemodilution (4,13), and other low-flow states (30). Emulsified PFCs have been shown to preserve systemic oxygen delivery in venous gas embolism (32) and, perhaps more significantly, enhanced oxygen delivery to injured neurologic tissue in several animal models (8,26). Since inert gas (most commonly N_2) released from supersaturated tissues leads to bubble formation, increasing inert gas elimination should be beneficial in treating DCS. In fact, certain PFC formulations have demonstrated enhanced nitrogen elimination (21,35). In cardiopulmonary bypass models PFCs have decreased bubble quantity and, potentially more important, bubble size (34).

In addition to enhanced gas transport, PFCs have other properties that may mitigate DCS. Bubbles themselves appear to lead to endothelial dysfunction, largely based on surface tension interactions (14). It appears

that the surfactant-like properties of PFC change bubble adhesion to the endothelium, thus causing less dysfunction (29) and less thrombin production (11). As both endothelial dysfunction and local clot formation would likely further decrease distal oxygen delivery, PFC should be beneficial in DCS.

Clearly, the oxygen delivery, gas eliminating, and surfactant properties of PFCs make them ideal candidates for DCS therapeutic agents. PFCs have a long history of mitigating the development of DCS (7,22). Small animal models have shown PFCs to prevent DCS since first published (22). The largest animal model previously studied was the 20-kg swine decompressed from a 22-h saturation at 4.9 ATA (9). In that study, animals received $6\ \text{cc} \cdot \text{kg}^{-1}$ of intravenous PFC (Oxygent®, Alliance Pharmaceutical, San Diego, CA) and oxygen, and a corticosteroid immediately after reaching normal atmospheric pressure (1 ATA). PFC significantly decreased cardiopulmonary DCS, delayed cardiopulmonary DCS onset, and completely prevented neurologic DCS. In that study, the average DCS onset time in treated and control groups was approximately 14 min, thus PFCs were employed as a preventive, not a therapeutic agent.

Dromsky's study was specifically designed to evaluate the use of PFC in a DISSUB scenario. In such a situation trapped submariners would likely be exposed to increased atmospheric pressure and need to rapidly decompress to surface pressure. Models exist to help predict the likelihood of DCS (25) and can be used to anticipate casualties. In high-risk exposures such as DISSUB the prophylactic use of PFC may make sense. However, DCS occurs even when decompression tables are followed (17) and given the overall low risk for DCS, preventive therapies are generally less warranted. Additionally, the physical realities of a DISSUB (escape/rescue logistics, sea state conditions, IV placement) make the immediate infusion of PFC unlikely.

Though this study is just one in a series demonstrating a benefit in DCS outcome, the fact that delayed administration of PFC was still beneficial is highly encouraging. We used the onset of cutis marmorata to trigger PFC (or saline) administration and oxygen. In developing this model, cutis incidence was associated with a 60% chance of death or paralysis (Mahon RT; unpublished data; 2008), which appears in line with results from our control group. Cutis marmorata onset in the swine model has been correlated with DCS severity (1,7,23) and has always preceded severe DCS. This study might be criticized for not waiting until the observable onset of severe DCS. However, in work-up experiments, we found it very difficult to maintain animal comfort at the onset of severe DCS and felt that both expeditious therapy and animal comfort could not be simultaneously addressed.

Unlike other studies, we incorporated a systematic evaluation of spinal cord dysfunction. Spinal cord injury models in swine are not as developed as in other species. We used the modified Tarlov scale as a gross measure of functional outcome. Clearly this is an imperfect scale in that animals with histology abnormalities maintained a preserved gait. More sophisticated measures of

spinal cord function were not used, but certainly may have enhanced the findings in this body of work (20). In conclusion, Oxycyte PFC ($5\text{cc} \cdot \text{kg}^{-1}$) administered after DCS onset decreased mortality. While this, the first trial to examine PFC post-dive and after DCS onset is promising, further study into the mechanisms of benefit and delayed DCS therapy are warranted.

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